

Life's transistors

Fred J. Sigworth

Voltage-gated ion channels control electrical activity in nerve, muscle and many other cell types. The crystal structure of a bacterial voltage-gated channel reveals the astonishingly simple design of its voltage sensor.

The membranes of living cells, from bacteria to humans, contain protein macromolecules that behave rather like field-effect transistors. In transistors, the flow of electrons through a semiconductor 'channel' is governed by the voltage applied to a 'gate' electrode. With the protein equivalents — voltage-gated ion channels — an appropriate voltage, imposed across the cell membrane, causes the channels to open and allows a current of ions to cross the membrane. The molecular structures within ion channels that sense the membrane voltage have remained obscure for the 50 years since Hodgkin and Huxley first described¹ their function. But the voltage sensors have at last been made visible, in the X-ray structure of a potassium ion channel. Youxing Jiang, Roderick MacKinnon and colleagues present this work on page 33 of this issue², and in a second paper (on page 42)³ they describe tests of a hypothesis for voltage-sensor motion.

The functional unit of a voltage-gated channel is an assembly of four proteins, or subunits; in each, the polypeptide chain snakes back and forth across the membrane six times. This 'six-transmembrane' structure is seen in the voltage-gated potassium, sodium and calcium channel families, and also in other channel types. As voltage-sensing devices, these channels can perform much better than their electronic counterparts (Fig. 1a). Their high sensitivity to voltage is important, because cellular voltage changes are small.

A simple biological voltage sensor would be a charged particle within a cell membrane, with an imposed voltage difference driving the particle from one surface of the membrane to the other. Theory shows that, to explain the observed sensitivity of voltage-gated channels, at least 12 elementary charges must participate in the sensing mechanism. Indeed, direct measurements of 'gating currents' confirm that the total charge displacement in a channel's voltage sensors is about 13 elementary charges per channel⁴.

So where in the channel protein are these charges found? It has been established⁵ that, in a six-transmembrane channel, the transmembrane segments known as S6 helices — one from each of the four subunits — form a 'gate'. That is, they form a bundle that can pinch off the ion pathway, effectively closing the channel. Meanwhile the fourth segment,

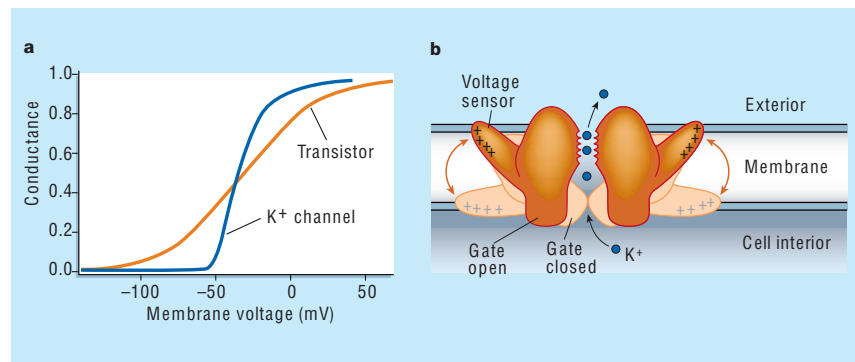


Figure 1 Voltage sensing in a potassium ion channel. a, The control of ion flow through voltage-gated channels is very sensitive to the voltage across the cell membrane. By comparison, an electronic device such as a transistor is much less sensitive to applied voltage. b, MacKinnon and colleagues^{2,3} have found that the voltage sensors in a bacterial potassium channel are charged 'paddles' that move through the fluid membrane interior. Four voltage sensors (two of which are shown here) are linked mechanically to the channel's 'gate'. Each voltage sensor has four tethered positive charges (arginine amino acids); the high sensitivity of channel gating results from the transport of so many charges, 16 in all, most of the way across the membrane.

S4, has always seemed a natural candidate for the voltage sensor that causes the gate to open and close⁶. Depending on the channel type, S4 has four to seven positive charges, mostly from arginine amino-acid residues. Moreover, S4 is otherwise very hydrophobic, which makes it likely to be embedded in the oily interior of a protein — or within the membrane itself. Because of these properties, S4 has been the subject of intense study⁷. Mutation analyses have shown it to be important in voltage sensing; spectroscopic probes have shown that it moves in response to voltage; and chemical-modification studies have revealed that some of its amino acids are alternately exposed on the internal or external face of the membrane, depending on the membrane voltage. So it has become clear that the four S4 segments per channel are the main voltage sensors.

What structural design would allow so many charges to move so far, crossing the 30-Å-thick, electrostatically hostile interior of a cell membrane? Practically everyone in the ion-channel field (including myself) has imagined the S4 segment to be an α -helix — a common structural feature of proteins, in which the polypeptide backbone is twisted into a spiral — that is packed snugly among the other helices of the protein. It has been thought that the S4 helix would undergo a shift or a rotation in response to voltage, and that charge transport might even be ampli-

fied by 'focusing' the membrane electric field near S4. This model has made its way into the textbooks. But the results of MacKinnon and colleagues show that it is almost certainly wrong.

Determination of the structure of a voltage-gated channel has been long in coming. The five-year effort in the MacKinnon laboratory involved trials of many channel proteins, none of which formed either two-dimensional or three-dimensional crystals. Reasoning that these failures might reflect a particularly loose protein structure, MacKinnon and colleagues decided to use parts of antibody molecules (so-called Fab fragments) as a scaffold to aid crystallization, and also chose a particularly rugged channel protein. Although its origin is the archaeobacterium *Aeropyrum pernix*, this protein, KvAP, has sequence features and electrical characteristics⁸ that place it firmly in the broad family of voltage-gated potassium channels.

The resulting X-ray structure² of KvAP shows the expected potassium channel core, consisting of transmembrane segments S5 to S6, surrounded by S1 through to part of S3. What was unexpected is that the S4 helix, along with the second part of S3, forms an α -helical hairpin — a 'paddle' that extends out from the channel core into the membrane's fluid interior (see Fig. 3 of ref. 2, page 35). The paddle has a flexible connection to the

rest of the channel, as the authors show by comparison with another crystal structure, of segments S1 to S4 alone. This flexibility explains the difficulty that the authors encountered in crystallizing the protein; it also suggests a mechanism for voltage sensing. The paddle is a hydrophobic, charged particle that can move in the membrane interior, transporting its four positive charges from one membrane surface to the other (Fig. 1b).

It is the location of S4 — not embedded in the protein core, but loose in the membrane — that is the big surprise here. It explains an old puzzle, that small lipid-soluble molecules somehow have ready access to ion-channel voltage sensors. Such molecules include local anaesthetics, the alkaloid nerve toxins and the well-known insecticides allethrin and DDT. It is now easy to imagine them diffusing up to the voltage-sensor paddle from within the lipid membrane interior.

An X-ray crystal structure is like a posed photograph; in the KvAP crystal, for instance, the voltage-sensor paddle is held firmly in place by an antibody scaffold. What can be learned about the paddle's natural conformation and movements? A few years ago, Horn and colleagues⁹ showed for sodium channels that a bulky moiety, attached by chemical modification to an S4 amino acid on the outside of the membrane, can actually be dragged through to the inner surface in response to an inside-negative voltage. In their second paper³, MacKinnon and colleagues show that a much larger molecule — biotin plus a 17-Å linker — flips across the membrane in a voltage-dependent manner when it is attached to an S4 amino acid in KvAP. They conclude that the S3–S4 paddle moves through a quite unrestricted space. They go on to attach this biotin–linker molecule to various other sites in the paddle, to map its position relative to the membrane surfaces at positive and negative voltages.

After all this, MacKinnon and co-workers have still left a few questions to be answered. The actual conformation of the channel in the membrane will need to be clarified, because in the crystal the membrane is replaced by a blanket of detergent molecules. Questions also remain about the disposition of the amino-terminal end of the protein (thought to be intracellular) and of the loop between the S3 and S4 segments in related channels (in the well-studied Shaker potassium channel, this loop is always accessible from the outside surface). Moreover, details of the motions of the voltage sensor — in some channels the charge movement occurs in several discrete steps — remain to be worked out, as does the energetic issue of moving the quadruply charged paddle through the membrane interior. But the structure of KvAP's voltage sensor, so simple and, with hindsight, so obvious, is a wonderful end to a 50-year-old mystery. ■

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Optics

Positively negative

John Pendry

An artificially created material with negative refractive index has opened the door to new phenomena — and controversy. New work finally sets the seal of experimental confirmation on negative refraction.

As light crosses a boundary between different materials (such as between air and water), its speed changes and it refracts, or bends. On entering most materials, light refracts with a positive angle, as shown in Fig. 1. But some years ago, Veselago¹ suggested that some materials might produce 'negative refraction': light would refract the other way, through a negative angle. In 2001, the observation of negative refraction was reported in an artificially created material² — but not everyone was convinced. Now two papers in *Physical Review Letters*, one by Parazzoli *et al.*³, the other by Houck *et al.*⁴, report experiments at radio frequencies that confirm the existence of negative refraction.

The response of a material to electric and magnetic fields is characterized by its permittivity, ϵ , and its permeability, μ , respectively. Veselago argued that if both ϵ and μ were negative, it follows that the refractive index of the material, which determines the velocity of light within it, would also be negative. There matters rested, because, although there are natural materials with negative ϵ , there are none with both negative ϵ and negative μ . In the absence of any materials on which to experiment, the concept was purely theoretical.

But the situation changed with the publi-

cation of three papers. In the first, my own group showed⁵ that it is easy to fabricate an artificial material with negative ϵ using a lattice of thin metal wires. We then showed⁶ how to do the same thing for μ : the required magnetic response was obtained from a lattice of metal 'split rings' that resonate with magnetic fields of a given frequency; the induced currents give a negative magnetic response. The third paper, by Smith and colleagues⁷, made the key advance. This team made a structure that combined these elements in a microwave experiment, the first demonstration of negative ϵ and negative μ in the same material. They went on to demonstrate negative refraction in their material².

This work sparked great interest in negative materials, but also a fair bit of controversy: one group of sceptics wrote⁸ of "Wave Refraction in Negative-Index Media: Always Positive...". Others claimed, unfairly in my view, that the results were an artefact of losses in the system and observations taken too close to the sample. The challenge to experimenters was to reproduce the results and eliminate all areas of doubt.

Parazzoli *et al.*³ and Houck *et al.*⁴ follow the same basic design for the negatively refracting material: split rings of copper are

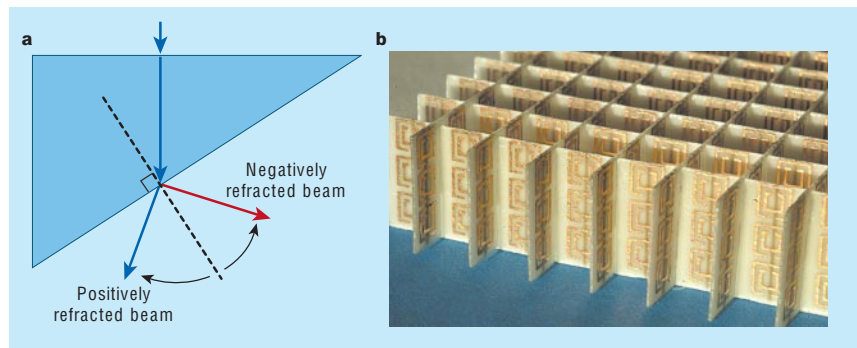


Figure 1 Negative refraction. a, Light incident on a normal material refracts at a positive angle (blue), but in a negative-index material the refraction angle is negative (red). Negative refraction occurs only in specially engineered materials. b, This arrangement of fibreglass sheets (1 cm high) in which is embedded an array of copper loops and wires was the first 'material' for which negative refraction was seen².